

Effect of Cold plasma treatment on seed quality parameters under cold stress in rice (*Oryza sativa* L.)

ABSTRACT

Abiotic stresses impinge rice production all over the world. Low temperature stress is one among the major abiotic stresses in crop plants significantly affecting plant growth, survival, reproduction, and dispersal. Therefore an experiment was carried out on rice variety BPT 5204 to study the effects of cold plasma treatment under cold stress. Attempt was made to standardize appropriate stage of exposure (dry seeds, sprouted seeds), voltage (30kv, 20kv) and duration of exposure (10min, 20min) based on the performance in vigour tests i.e., germination, seedling dry weight, seedling vigour index-II, field emergence test, abnormality and imbibition tests at a temperature of 18°C. Results indicated that exposure of sprouts of BPT 5204 at 20kv for 20min has given highest germination (88%), seedling dry weight (68.10 mg), seedling vigour index II (597), field emergence (77%), imbibition rate (0.495) and lowest abnormality (12%) in comparison to control and other treatments. This indicates that cold plasma treated at 20kv for 20min on paddy sprout had a greater stimulatory effect on plant growth and development under cold temperature conditions.

1. INTRODUCTION

Rice (*Oryza sativa* L.), second among the major cereals worldwide, is considered as a life sustaining crop for majority of the population in the world. It is a staple food for half of the world's population providing dietary energy and protein up to 75% to almost 2.5 billion people of the world. Globally, rice output is expected to be 527.6 million tons for the year 2024-25, which is 2% less than the previous year (Paddy Outlook-June 2024 PJTSAU). Rice output is expected to be 138.00 million tonnes (www.usda.gov) in India and 82.80 lakh tonnes in Telangana in 2023-24 (www.agri.telangana.gov.in).

In spite of drastic increase in rice production in the past decades due to development of high yielding, pest and disease resistant varieties, around 800 million people especially belonging to different countries suffer from hunger (Fahad *et al.*, 2018). Therefore more rice is to be produced within the available resources to feed the increasing population. This necessitates development of cultivars or techniques to perform better even under yield limiting situations

especially in the current era of climate change. Rice production is negatively impacted by both biotic and abiotic stressors in various parts of the world. Biotic variables include insects, pests, and diseases, while drought, high or low temperatures, salinity, pollution, and soil pH allude to abiotic stresses (Bashier *et al.*, 2007; Oerke *et al.*, 2001). Although rice is adaptable to a wide range of climatic conditions, environmental factors like temperature and radiation can show profound effect on rice production (Fahad *et al.*, 2015). Rice is a temperature-responsive crop, and low temperatures significantly diminish its yield (Lou *et al.*, 2007; Howarth and Ougham 1993). At early growth stages, low temperature stress halts seedling establishment and eventually leads to unpredictable and non-uniform crop maturation (Lou *et al.*, 2007). Sensitivity of rice crop to cold stress during reproductive and seedling stages leads to poor germination, stunted seedlings, yellowing or withering, and diminished tillering (Mukhopadhyay *et al.*, 2004).

Cold plasma is considered as the fourth state of matter, with unique properties. It is an ionized gas made up of excited atoms, molecules, and ions that coexist with reactive species such as electrons, negative and positive ions, free radicals, atoms, and molecules in their excited or ground states. Because of its non-equilibrium state, the gas survives at low temperatures through the cooling of ions and uncharged molecules rather than electron energy transfer (Bourke *et al.*, 2018). The gas can be ionized by applying thermal energy (heating) or electromagnetic fields (electric fields or high energy light). The use of an electrical field is the most well-known way for technical applications of plasma at ambient temperature and atmospheric pressure. In this sense, cold does not refer to sub-zero temperatures, nor does a temperature range. Plasma sources operating at temperatures below 60 °C are commonly referred to as cold plasmas (Misra *et al.*, 2019a,b). Cold plasma discharge is controlled by the Townsend-based ionization avalanche mechanism. As a high voltage current is delivered, liberated electrons speed and collide with neutral gas atoms. As a result, the collision causes the gas to be ionized. As the process progresses, electron and ion avalanches form in the gas gap, with ions being accelerated to the cathode (ground electrode) and electrons being accelerated to the anode (high voltage electrode). The high intensity of the avalanches causes the ion avalanches to release electrons from the ground electrode, triggering the release of self-sustaining gas as the phase approaches the breakdown voltage (Wagner *et al.*, 2003).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been identified as the most active plasma components capable of inducing oxidation, and these species are

primarily responsible for organic compound degradation or microorganism inactivation (Laroussi and Leipold, 2004; Surowsky *et al.*, 2015). Because of their extreme reactivity, ROS and RNS can react with all cell components, breaking chemical bonds and causing cell membrane damages. As a result, most reactive species help to etch and eventually distribute macromolecules within cells (Abd El-Aziz *et al.*, 2014; Park *et al.*, 2015; Yang *et al.*, 2016). ROS and RNS can be employed to give critical macronutrients to the targeted rice grain, resulting in the inactivation and decontamination of pathogenic fungus, thereby halting the formation of mycotoxins (Khamsen *et al.*, 2016).

In recent years, cold plasma has emerged as a viable alternative, particularly for post-harvest treatment management (Misna *et al.*, 2022). The use of non-equilibrium atmospheric pressure plasma (cold plasma) in biology has sparked widespread interest, particularly in health, agriculture, and fisheries. Plasma treatment has use in agriculture at several stages of production, including pre-planting, pre-harvest, and postharvest. Previous research has shown that cold plasma can be used to induce protein synthesis, promote seed germination and plant growth, sterilize/disinfect, and store foods. Numerous researchers have recently studied the use of cold plasma in increasing the growth of a variety of plants, including rice, wheat, soybean, sunflower, pea, radish, barley, tomato and Chinese cabbage (Hashizume *et al.*, 2021). In this pretext, the current study highlights the role and potential of cold plasma as a cold tolerance medium for enhancement of rice seed quality under low temperature conditions.

2. MATERIALS AND METHODS

The experiment was conducted on rice variety BPT 5204 for standardization of cold plasma treatment given at different voltages (20kv, 10kv), duration of exposure (10min, 20min) and stages of exposure (dry seeds, sprouted seeds). The best cold plasma treated seed was identified through laboratory vigour tests i.e., seed germination, seedling dry weight, seedling vigour index-II, field emergence test (FET), abnormality and imbibition rate carried out under cold conditions (18°C) and normal temperature conditions (control).

2.1 Germination (%)

The germination test was conducted as per the ISTA rules (2019) by employing between paper method. The test was conducted in three replications of 100 seeds each per treatment. The seeds were placed on the germination papers which were then rolled and placed in the walk-in germinator maintained at a constant temperature of $25 \pm 0.5^\circ\text{C}$ and more than 90

percent relative humidity. On the day of final count i.e., fourteenth day, the number of seeds germinated was counted and the per cent germination was calculated as follows:

$$\text{Germination \%} = \frac{\text{Number of total germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

2.2 Seedling dry weight (mg)

Ten normal seedlings from each replication were placed in butter paper bags and kept in an oven maintained at $80 \pm 1^\circ \text{C}$ for 24 hours. The oven dried seedlings were then placed in a desiccator for cooling. Weights of the dried seedlings along with their mean weights were recorded and expressed in milligrams per seedling.

2.3 Seedling vigour index II

The seedling vigour indices were calculated as per the formula suggested by Abdul Baki and Anderson (1973) as given below and expressed in whole number.

$$\text{Seedling vigour index II} = \text{Germination (\%)} \times \text{Seedling dry weight (mg)}$$

2.4 Field emergence (%)

Field emergence potential of seeds was measured as per the method suggested by Shenoy *et al.* (1990). Three replications of hundred seeds each were sown on a raised bed of red loamy soil with a spacing of 10cm between the rows. The number of seeds germinated in each row was counted on 14th day and percentage field emergence was calculated by using the formula:

$$\text{Field emergence (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.5 Abnormality (%)

The test was conducted in three replications of 100 seeds each per treatment. The seeds were placed on the germination papers which were then rolled and placed in the walk-in germinator maintained at a constant temperature of $25 \pm 0.5^\circ \text{C}$ and more than 90 percent relative humidity. On the day of final count i.e., fourteenth day, the number of abnormal seeds were counted and the per cent abnormality was calculated as follows:

$$\text{Abnormality \%} = \frac{\text{Number of abnormal seedlings}}{\text{Total number of seeds tested}} \times 100$$

2.6 Water Imbibition rate

Three replicates of 25 seeds each were weighed and then soaked in distilled water in a beaker. After 12 hrs the seeds were taken out and weights were recorded. The seeds were again put in the beaker and the weights were recorded at 2 hr intervals upto 40 hrs. Before weighing, the surface water of seeds was removed by gently pressing between two layers of filter paper, as described by Al-Mudayris and Jutzi, 1998. The imbibition rate was determined based on their weights and depicted in the graphical form.

3. RESULTS AND DISCUSSIONS

The results of ANOVA indicated presence of significant variation among all the factors (stages of exposure and treatments). The interaction effects also revealed significant variation for all the traits studied except seedling dry matter (cold and normal conditions) and field emergence (normal conditions) (Table 1).

3.1 Germination (%)

The data pertaining to germination was recorded and presented in Table 2. Under cold conditions, highest germination was recorded in T3(20kv, 20min) for both dry seed (81%) and sprouts (88%). Lowest germination was recorded by T4 (20kv, 10 min) for dry seed (71%) and both T1 (30kv, 20min) and T2 (30kv, 10min) for sprouts (82%). Further, it was observed that cold plasma treated sprouts recorded highest germination of 88% (T3-20kv, 20min) compared to treated dry seeds which showed highest germination of 81% (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 11.1% germination over the control while in the sprouts (T3) there is an increase of 9.0% over the control.

Under normal conditions, highest germination was recorded by T3 (20kv for 20min) for both dry seed (88%) and sprouts (92%). Lowest germination was recorded by both T1(30kv for 20 min) and T2 (30kv for 10 min) for dry seed (80%) and T1 (30kv for 20min) for sprouts (83%). Among the stages, treated sprouts recorded the highest germination of 92% (T3-20kv, 20min) compared to treated dry seeds (88%) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 9.0 % over the control while in sprouts (T3) there is an increase of 4.3% over the control. Thus the results indicated that cold plasma treatment significantly increased seed germination especially in dry seed when compared to control. Seeds contain a thin lipid layer on seed coat that makes the seeds water repellent and also reduces the strength of biopolymer chain that makes up the seed coat. Cold plasma treatment is effective in removal of this lipid layer thus enabling better water uptake through

seed coat and thereby improving germination. Similar findings were reported by Selcuk *et al.*(2008) and Seraet *al.* (2010).

3.2 Seedling dry weight (mg)

Under cold conditions, highest seedling dry weight was recorded in T3 (20kv, 20min) for both dry seed (64.97mg) and sprouts (68.10mg). Lowest seedling dry weight was recorded by T1 (30kv, 20 min) for dry seed (56.20mg) and T1(30kv, 20min) in sprouts (61.63mg). Among the stages, treated sprouts recorded the highest seedling dry weight of 68.10mg (T3-20kv, 20min) compared to treated dry seed (64.97 mg) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 12.8% over the control while in sprouts (T3) there is an increase of 8.7% over the control (Table 3).

Under normal conditions, highest seedling dry weight was recorded by T3(20kv, 20min) for both dry seed (74.27mg) and sprouts (77.40mg). Lowest seedling dry weight was recorded in T1(30kv, 20 min) for dry seed (64.07) and for T1(30kv, 20min) for sprouts (67.70). Further, it was observed that treated sprouts recorded the highest seedling dry weight of 77.40mg (T3-20kv, 20min) compared to treated dry seed (74.27 mg) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 14.1% over the control while in sprouts (T3) there is an increase of 12.4% over the control. The results revealed that seedling dry weight is found to be increased when the seeds are treated with cold plasma. It has been proposed that seedling dry weight is significantly related to seed reserve mobilization and the seed reserve depletion percentage. The amount of reserved food material mobilized during seedling growth ultimately contributed to difference in seedling dry weight. Cold plasma can induce the production of reactive oxygen species (ROS) in plants, which at controlled levels, can act as signalling molecules to enhance stress resistance. This helps seedlings cope better with environmental stresses, leading to improved growth and biomass accumulation. These results are in accordance with Perez-Piza *et al.*(2019) and Li *et al.* (2016).

3.3 Seedling vigour index II

The data on seedling vigour index II (SVI II) was recorded and presented in Table 4. Under cold conditions, highest seedling vigour index II was recorded in T3 (20kv, 20min) for both dry seed (524) and sprouts (597). Lowest seedling vigour index II was recorded by T1(30kv, 20 min) for dry seed (407) and for T1(30kv, 20min) for sprouts (503). Among the stages, sprouts recorded the highest seedling vigour index II of 597 (T3-20kv, 20min) compared to treated dry seed (524) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an

increase of 22.5% over the control while in sprouts (T3) there is an increase of 16.2% over the control.

Under normal conditions, highest seedling vigour index II was recorded in T3(20kv, 20min) for both dry seed (651) and sprouts (709). Lowest seedling vigour index II was recorded by T1 (30kv, 20 min) for dry seed (513) and for T1 (30kv, 20min) for sprouts (564). Further, it was observed that treated sprouts recorded the highest seedling vigour index II of 709 (T3-20kv, 20min) compared to treated dry seed (651) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 21.3% over the control while in sprouts (T3) there is an increase of 15.7% over the control. The results indicated that cold plasma treatment has a positive effect on seedling vigour index II. Cold plasma can induce a controlled increase in reactive oxygen species (ROS) within the seed. While excessive ROS can be harmful, a moderate increase can act as signaling molecules, promoting growth and development processes. Plasma treated seeds have better permeability, which lead to enhanced seed germination resulting in increased seedling vigour index-I and II (Azharonoket *et al.*, 2014).

3.4 Field emergence test (%)

The data related to field emergence is given in the Table 5. Under cold conditions, highest field emergence was recorded in T3(20kv, 20min) for both dry seed (70%) and sprouts (77%). Lowest field emergence was recorded by T1(30kv, 20 min) for dry seed (61%) and T2 (30kv,10min) for sprouts (66%). Among the stages, treated sprouts recorded the highest field emergence of 77% (T3-20kv, 20min) compared to treated dry seed (70%) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 12.8% over the control while in sprouts (T3) there is an increase of 5.1% over the control.

Under normal conditions, highest field emergence was recorded in T3 (20kv, 20min) for both dry seed (87%) and sprouts (91%). Lowest field emergence was recorded by T4(20kv, 10 min) for both dry seed (80%) and sprouts (85%). Further, it was observed that treated sprouts recorded the highest field emergence of 91% (T3-20kv, 20min) compared to treated dry seed (87%) (T3-20kv, 20min). The best treatment in dry seed (T3) showed an increase of 5.7% over the control while in sprouts (T3) there is an increase of 5.4% over the control. The results indicated that cold plasma treatment showed a stimulatory effect on emergence of seedlings in field conditions (Lofty *et al.* 2017). The reactive species generated by cold plasma at atmospheric or low pressure is involved in changing and activating the physical and chemical

properties, physiology, and biochemical and molecular processes in plants, which enhances germination, growth, and development in field conditions.

3.5 Abnormality (%)

The data about abnormality is presented in the Table 6. Under cold conditions, lowest abnormality was recorded in T3(20kv, 20min) both for dry seed (19%) and sprouts (12%). Highest abnormality was recorded by T4(20kv, 10 min) for dry seed (29%) and both T1 (30kv, 20min) and T2 (30kv, 10min) for sprouts (18%). Among the stages, treated sprouts recorded the lowest abnormality of 12% (T3-20kv, 20min) compared to treated dry seed (19%) (T3-20kv, 20min). The best treatment in dry seed (T3) showed a decrease of 32.1% over the control while in sprouts (T3) there is a decrease of 40% over the control.

Under normal conditions, lowest abnormality was recorded in T3 (20kv, 20min) for both dry seed (12%) and sprouts (8%). Highest abnormality was recorded by both T1 (30kv, 20 min) and T2 (30kv, 10min) for dry seed (20%) and T1 (30kv, 20min) for sprouts (17%). Among the stages, treated sprouts recorded the lowest abnormality of 8% (T3-20kv, 20min) compared to treated dry seed (12%) (T3-20kv, 20min). The best treatment in dry seed (T3) showed a decrease of 40% over the control while in sprouts (T3) there a decrease of 33.3% over the control. The result revealed that less percent of abnormal seedlings were seen in the plasma-treated sample and the percentage of abnormal seedlings varied significantly between the treated and non-treated. Plasma treatments had an antifungal impact on the pathogen, resulting in the formation of more normal seedlings and fewer abnormal seedlings. Plasma derived reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been powerful oxidizing agents that can penetrate microorganisms and modify their cell processes, rendering them inactive and perhaps leading to the decontamination of seed-borne pathogens. Similar findings were reported by Ramirez *et al.* (2017) and Klampfle *et al.* (2012).

3.6 Imbibition rate

The data on imbibition was depicted in the graphical form (Fig 1). The imbibition rate of seeds was determined by increase in weight and time. The highest imbibition was recorded in T3 (20kv, 20min) (0.495) and lowest imbibition was recorded in T1 (30kv, 20min) (0.482). The best treatment (T3) showed an increase of 3.2% over the control. The results indicated that cold plasma treatment has improved the imbibition rate of seeds. Seeds immersed into cold plasma are subjected to an attack by oxygen radicals and are bombarded by ions resulting in seed coat erosion and etched/eroded surfaces. The altered seed coat could

increase the hydrophilic ability of the seed, and eventually improve the water uptake of the seed. These results are in agreement with the findings of Bormashenko *et al.* (2012).

CONCLUSION

In the present study, it was found that cold plasma treatment of paddy seed at 20kv for 20mins has resulted in highest germination, seedling dry weight, seedling vigour index II, FET, imbibition rate and lowest abnormality percent compared to control and other treatments. Sprouted seed exhibited highest germination and other seed quality traits compared to dry seed, although the enhancement of these parameters compared to control was higher in dry seed. This study thus marks the profound positive effect of cold plasma treatment in improving the germination and vigour of paddy seed under unfavourable cold temperature conditions. These results may be further confirmed under field conditions.

Table 1: Analysis of variance (ANOVA)

	DF	Germination (%)		Seedling dry matter (mg)		Seedling vigour index II		Field emergence (%)		Abnormality (%)	
		Cold conditions	Normal conditions	Cold conditions	Normal conditions	Cold conditions	Normal conditions	Cold conditions	Normal conditions	Cold conditions	Normal conditions
S	1	580.80***	326.70***	156.40***	87.39***	61191.79***	41611.96***	425.63***	202.80***	580.80***	326.70***
T	4	60.59***	50.39***	58.06***	107.48***	11476.07***	18592.46***	66.71***	32.63***	60.59***	50.39***
SxT	4	16.89***	12.61***	1.47	0.37	777.67***	534.91*	47.21***	2.97	16.89***	12.61***
Error	20	0.67	1.20	1.29	1.50	88.80	163.50	0.77	1.10	0.67	1.20
Total	29	31.18	20.79	14.49	18.92	3861.48	4185.90	30.92	12.67	31.18	20.79
General mean		79.00	85.10	62.00	69.20	491.58	590.33	67.90	85.60	21.00	14.90
C.V		1.03	1.29	1.82	1.77	1.92	2.17	1.29	1.23	3.89	7.36
C.D @ 95%											
Si x Sj		0.62	0.83	0.86	0.93	7.18	9.74	0.67	0.80	0.62	0.83
Ti x Tj		0.98	1.32	1.37	1.48	11.35	15.40	1.05	1.26	0.98	1.32
SiTi x SjTj		1.39	1.87	1.93	2.09	16.05	21.78	1.49	1.79	1.39	1.87

*** indicates significant variation.

Table 2: Germination % in rice when exposed to different treatments under both conditions

Treatments	Germination (%)					
	Cold conditions (18°C)			Normal conditions		
	Dry seed	Sprouts	Mean	Dry seed	Sprouts	Mean
30kv 20min (T1)	72 (58.27)	82 (64.65)	77 (61.46)	80 (63.44)	83 (65.91)	82 (64.67)
30kv 10min (T2)	77 (61.35)	82 (64.90)	80 (63.12)	80 (63.21)	90 (71.58)	85 (67.39)
20kv 20min (T3)	81 (63.92)	88 (69.46)	84 (66.69)	88 (69.46)	92 (73.23)	90 (71.35)
20kv 10min (T4)	71 (57.63)	85 (67.48)	78 (62.56)	81 (64.41)	89 (70.64)	85 (67.53)
Control (T5)	72 (57.84)	80 (63.68)	76 (60.76)	80 (63.68)	88 (69.74)	84 (66.71)
Mean	75 (59.80)	83 (66.00)		82 (65)	88 (70.20)	
CV%	1.03% (0.99 %)			1.29% (1.31%)		
	SE (m)	CD		SE (m)	CD	
S	0.21 (0.16)	0.62 (0.48)		0.28 (0.23)	0.83 (0.68)	
T	0.33 (0.26)	0.98 (0.75)		0.45 (0.36)	1.32 (1.07)	
S×T	0.47 (0.36)	1.39 (1.07)		0.63 (0.51)	1.87 (1.52)	

Table 3: Seedling dry weight of rice obtained from different treatments under both conditions

Treatments	Seedling dry weight (mg)					
	Cold conditions			Normal conditions		
	Dry seed	Sprouts	Mean	Dry seed	Sprouts	Mean
30kv 20min (T1)	56.20	61.63	58.92	64.07	67.70	65.88
30kv 10min (T2)	59.70	64.00	61.85	69.43	72.20	70.82
20kv 20min (T3)	64.97	68.10	66.53	74.27	77.40	75.83
20kv 10min (T4)	61.13	65.53	63.33	65.97	69.43	67.70
Control (T5)	56.60	62.17	59.38	63.73	67.80	65.77
Mean	59.70	64.30		67.50	70.90	
CV%	1.83%			1.77%		
	SE (m)	CD		SE (m)	CD	
S	0.29	0.86		0.32	0.93	
T	0.46	1.37		0.50	1.48	
S×T	0.65	1.93		0.71	2.09	

Table 4: Seedling vigour index II of rice obtained from different treatments under both conditions

Treatments	Seedling vigour index II					
	Cold conditions			Normal conditions		
	Dry seed	Sprouts	Mean	Dry seed	Sprouts	Mean
30kv 20min (T1)	407	503	455	513	564	538
30kv 10min (T2)	460	525	492	553	650	602
20kv 20min (T3)	524	597	561	651	709	680
20kv 10min (T4)	436	559	498	537	618	577
Control (T5)	406	500	453	512	597	554
Mean	446	537		553	628	
CV%	1.92%			2.17%		
	SE (m)	CD		SE (m)	CD	
S	2.43	7.18		3.30	9.74	
T	3.85	11.35		5.22	15.40	
S×T	5.44	16.05		7.38	21.78	

Table 5: Field emergence of rice seedlings when exposed to different treatments under both conditions

Treatments	Field emergence (%)					
	Cold conditions (18°C)			Normal conditions		
	Dry seed	Sprouts	Mean	Dry seed	Sprouts	Mean
30kv 20min (T1)	61 (51.55)	75 (60.00)	68 (55.78)	83 (65.66)	88 (69.45)	85 (67.55)
30kv 10min (T2)	62 (51.75)	66 (54.53)	64 (53.14)	83 (65.66)	91 (72.25)	87 (68.95)
20kv 20min (T3)	70 (56.58)	77 (61.12)	73 (58.85)	87 (68.60)	91 (72.56)	89 (70.58)
20kv 10min (T4)	67 (55.14)	67 (55.15)	67 (55.14)	80 (63.68)	85 (67.48)	83 (65.58)
Control (T5)	61 (51.16)	73 (58.70)	67 (54.93)	82 (64.90)	86 (68.31)	84 (66.60)
Mean	64 (53.20)	72 (57.90)		83 (65.7)	88 (70.0)	
CV%	1.29% (0.98 %)			1.23% (1.33 %)		
	SE (m)	CD		SE (m)	CD	
S	0.23 (0.14)	0.67 (0.41)		0.27 (0.23)	0.80 (0.69)	
T	0.36 (0.22)	1.05 (0.65)		0.43 (0.37)	1.26 (1.09)	
S×T	0.51 (0.31)	1.49 (0.92)		0.61 (0.52)	1.79 (1.55)	

Table 6: Abnormality in rice seeds obtained from different treatments under both conditions

Treatments	Abnormality (%)					
	Cold conditions (18°C)			Normal conditions		
	Dry seed	Sprouts	Mean	Dry seed	Sprouts	Mean
30kv 20min (T1)	28 (31.73)	18 (25.35)	23 (28.54)	20 (26.56)	17 (24.09)	18 (25.33)
30kv 10min (T2)	23 (28.65)	18 (25.10)	21 (26.88)	20 (26.79)	10 (18.42)	15 (22.61)
20kv 20min (T3)	19 (26.08)	12 (20.54)	16 (23.31)	12 (20.54)	8 (16.77)	10 (18.65)
20kv 10min (T4)	29 (32.37)	15 (22.52)	22 (27.44)	19 (25.59)	11 (19.36)	15 (22.47)
Control (T5)	28 (32.16)	20 (26.32)	24 (29.24)	20 (26.32)	12 (20.26)	16 (23.29)
Mean	25 (30.2)	17 (24.0)		18 (25.2)	12 (19.8)	
CV%	3.89% (2.31 %)			7.36% (3.96%)		
	SE (m)	CD		SE (m)	CD	
S	0.21 (0.16)	0.62 (0.48)		0.28 (0.23)	0.83 (0.68)	
T	0.33 (0.26)	0.98 (0.75)		0.45 (0.36)	1.32 (1.07)	
S×T	0.47 (0.36)	1.39 (1.07)		0.63 (0.51)	1.87 (1.52)	

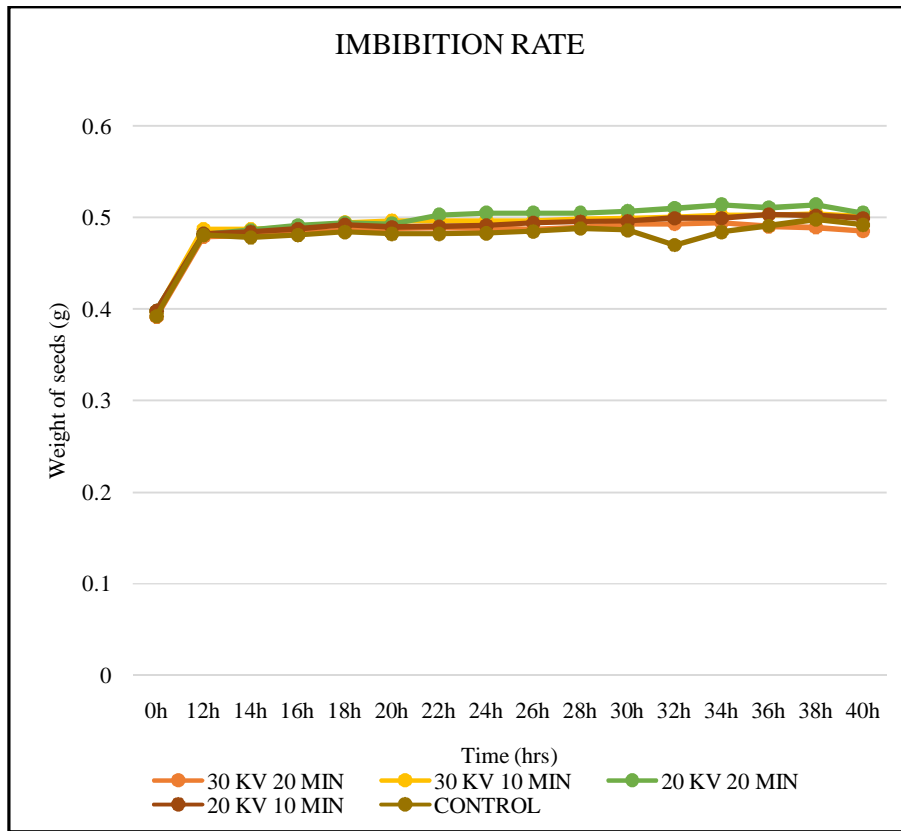


Fig 1: Imbibition rate of seeds in different treatments

REFERENCES:

- Abd El-Aziz, M.F., Mahmoud, E.A. and Elaragi, G.M., 2014. Non thermal plasma for control of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). *Journal of stored products research*, 59, pp.215-221.
- Abdul Baki, A.A. and Anderson, J.D., 1973. Vigor determination in soybean seed by multiple criteria 1. *Crop science*, 13(6), pp.630-633.
- Al-Mudaris, M.A. and Jutzi, S.C., 1998. The influence of genotype, priming material, temperature and osmotic potential of priming solution on imbibition and subsequent germination of sorghum and pearl millet seeds during and after treatment. *Der Tropenlandwirt-Journal of Agriculture in the Tropics and Subtropics*, 99(2), pp.133-145.
- Azharonok, V., Filatova, I., Shedikova, O. and Shik, A., 2011, March. Fungicidal and bactericidal effect of plasma and radiowave treatment on biological and medical materials. In *NATO Advanced Research Workshop "Plasma for biodecontamination medicine and food security," Jasna (Slovakia)* (pp. 95-96).
- Bashir, K., Khan, N.M., Rasheed, S. and Salim, M., 2007. Indica rice varietal development in Pakistan: an overview. *Paddy and Water Environment*, 5, pp.73-81.
- Bormashenko, E., Grynyov, R., Bormashenko, Y. and Drori, E., 2012. Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds. *Scientific reports*, 2(1), p.741.
- Bourke, P., Ziuzina, D., Boehm, D., Cullen, P.J. and Keener, K., 2018. The potential of cold plasma for safe and sustainable food production. *Trends in biotechnology*, 36(6), pp.615-626.
- Fahad, S., Hussain, S., Saud, S., Tanveer, M., Bajwa, A.A., Hassan, S., Shah, A.N., Ullah, A., Wu, C., Khan, F.A. and Shah, F., 2015. A biochar application protects rice pollen from high-temperature stress. *Plant Physiology and Biochemistry*, 96, pp.281-287.
- Fahad, S., Ihsan, M.Z., Khaliq, A., Daur, I., Saud, S., Alzamanan, S., Nasim, W., Abdullah, M., Khan, I.A., Wu, C. and Wang, D., 2018. Consequences of high temperature under changing climate optima for rice pollen characteristics-concepts and perspectives. *Archives of Agronomy and Soil Science*, 64(11), pp.1473-1488.

Gomez-Ramírez, A., Lopez-Santos, C., Cantos, M., García, J.L., Molina, R., Cotrino, J., Espinos, J.P. and Gonzalez-Elipe, A.R., 2017. Surface chemistry and germination improvement of Quinoa seeds subjected to plasma activation. *Scientific reports*, 7(1), p.5924.

Hashizume, H., Kitano, H., Mizuno, H., Abe, A., Yuasa, G., Tohno, S., Tanaka, H., Ishikawa, K., Matsumoto, S., Sakakibara, H. and Nikawa, S., 2021. Improvement of yield and grain quality by periodic cold plasma treatment with rice plants in a paddy field. *Plasma Processes and Polymers*, 18(1), p.2000181.

Hoon Park, J., Kumar, N., Hoon Park, D., Yusupov, M., Neyts, E.C., Verlact, C.C., Bogaerts, A., Ho Kang, M., Sup Uhm, H., Ha Choi, E. and Attri, P., 2015. A comparative study for the inactivation of multidrug resistance bacteria using dielectric barrier discharge and nano-second pulsed plasma. *Scientific reports*, 5(1), p.13849.

Howarth, C.J. and Ougham, H.J., 1993. Gene expression under temperature stress. *New Phytologist*, 125(1), pp.1-26.

ISTA. 2019. International Rules for Seed Testing, International Seed Testing Association, Bassersdorf, Switzerland.

Khamsen, N., Onwimol, D., Teerakawanich, N., Dechanupaprittha, S., Kanokbannakorn, W., Hongesombut, K. and Srisophonphan, S., 2016. Rice (*Oryza sativa* L.) seed sterilization and germination enhancement via atmospheric hybrid nonthermal discharge plasma. *ACS applied materials & interfaces*, 8(30), pp.19268-19275.

Klämpfl, T.G., Isbary, G., Shimizu, T., Li, Y.F., Zimmermann, J.L., Stolz, W., Schlegel, J., Morfill, G.E. and Schmidt, H.U., 2012. Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. *Applied and environmental microbiology*, 78(15), pp.5077-5082.

Laroussi, M. and Leipold, F., 2004. Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*, 233(1-3), pp.81-86.

Li, L., Li, J., Shen, M., Hou, J., Shao, H., Dong, Y. and Jiang, J., 2016. Improving seed germination and peanut yields by cold plasma treatment. *Plasma Science and Technology*, 18(10), p.1027.

Lotfy, K., 2017. Effects of cold atmospheric plasma jet treatment on the seed germination and enhancement growth of watermelon. *Open Journal of Applied Sciences*, 7(12), p.705.

Lou, Q., Chen, L., Sun, Z., Xing, Y., Li, J., Xu, X., Mei, H. and Luo, L., 2007. A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.). *Euphytica*, 158, pp.87-94.

Misnal, M.F.I., Redzuan, N., Zainal, M.N.F., Ahmad, N., Ibrahim, R.K.R. and Agun, L., 2022. Cold plasma: A potential alternative for rice grain postharvest treatment management in Malaysia. *Rice Science*, 29(1), pp.1-15.

Misra, N.N., Yadav, B., Roopesh, M.S. and Jo, C., 2019a. Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. *Comprehensive reviews in food science and food safety*, 18(1), pp.106-120.

Misra, N.N., Yopez, X., Xu, L. and Keener, K., 2019b. In-package cold plasma technologies. *Journal of Food Engineering*, 244, pp.21-31.

Mukhopadhyay, A., Vij, S. and Tyagi, A.K., 2004. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proceedings of the National Academy of Sciences*, 101(16), pp.6309-6314.

Oerke, E.C. and Dehne, H.W., 2004. Safeguarding production—losses in major crops and the role of crop protection. *Crop protection*, 23(4), pp.275-285.

Paddy Outlook, June. 2024. Agriculture Market Intelligence Centre, PJTSAU.

Pérez-Pizá, M.C., Prevosto, L., Grijalba, P.E., Zilli, C.G., Cejas, E., Mancinelli, B. and Balestrasse, K.B., 2019. Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status. *Heliyon*, 5(4).

Production of Rice in India. 2023. www.usda.gov.

Production of Rice in Telangana. 2023. www.agri.telangana.gov.in.

Selcuk, M., Oksuz, L. and Basaran, P., 2008. Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillium* spp. by cold plasma treatment. *Bioresource technology*, 99(11), pp.5104-5109.

Sera, B., Spatenka, P., Šerý, M., Vrchotova, N. and Hruskova, I., 2010. Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Transactions on Plasma Science*, 38(10), pp.2963-2968.

Shenoy, V.V., Dadlani, M. and Seshu, D.V., 1990. Association of laboratory assessed parameters with field emergence in rice: the nonanoic acid stress as a seed vigour test.

Surowsky, B., Schlüter, O. and Knorr, D., 2015. Interactions of non-thermal atmospheric pressure plasma with solid and liquid food systems: a review. *Food Engineering Reviews*, 7, pp.82-108.

Wagner, H.E., Brandenburg, R., Kozlov, K.V., Sonnenfeld, A., Michel, P. and Behnke, J.F., 2003. The barrier discharge: basic properties and applications to surface treatment. *Vacuum*, 71(3), pp.417-436.

Yang, X., Chang, X., Tei, R. and Nagatsu, M., 2016. Effect of excited nitrogen atoms on inactivation of spore-forming microorganisms in low pressure N₂/O₂ surface-wave plasma. *Journal of Physics D: Applied Physics*, 49(23), p.235205.

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